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Effect of Disulfiram on Viability and Proliferation of Virus Transformed Rat Sarcoma Cells

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The aim of our study was to evaluate the influence of disulfiram (Antabuse) on viability and proliferation of cultured retrovirus-transformed rat sarcoma cells (permanent cell line LSR-SF-SR). The investigations were performed by thiazolyl blue tetrazolium bromide (MTT) test, crystal violet staining, double staining with acridine orange and propidium iodide in short-term (24-72h) experiments with monolayer cultures as well as in long-term experiments (30 days) by 3D-colony forming method. The results obtained reveal that applied at a concentration range of 0.3 –100 μg/ml disulfiram expresses significant cytotoxic effect that is time- and concentration- dependent.

Key words: disulfiram, Rous sarcoma virus, rat sarcoma, cell culture, cytotoxicity

Introduction

Disulfiram (DS), an FDA (Food and Drug administration, USA) approved drug for the treatment of alocoholism has been also reported to express promising antitumor activity in cellular and animal models as well as in humans [7, 10]. According to the literature available, in this study we report for the first time data about inhibitory effect of disulfiram on 2D- and 3D-growth of cultured LSR-SF-SR rat sarcoma cells transformed by Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV). The cells contain *v-src* gene - the cellular analogues of this gene are known to be involved (when their expression and functioning are disturbed) in pathogenesis of various malignancies in humans and animals [9, 11].

Materials and Methods

Tetraethylthiuram disulfide (Disulfiram) was provided by Sigma-Aldrich Co (Germany, Lot #BCBN4962V). Disulfiram was dissolved in dimethylsulfoxide (DMSO – the

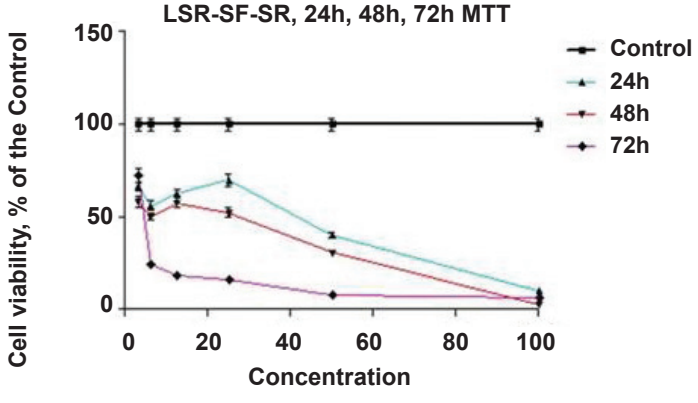
concentration of DS in the stock solution was 1 mg/ml containing 2 % DMSO) and then diluted in culture medium.

Permanent cell line LSR-SF-SR established from a transplantable sarcoma in rat, induced by Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV) [1] was used as an experimental model in our study. RSV, as well as cells transformed by the virus, contain *v-src* gene [11]. The influence of disulfiram on cell viability and proliferation was studied as it was previously described [2, 4], in short-term (24-72h, with monolayer cultures) experiments by MTT test (MTT), crystal violet staining (CV) and double staining with acridine orange and propidium iodide (AO/PI) as well as in long-term experiments (30 days) with 3D-colony-forming method (CFM). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test and Origin 6.1™ .

Results

The results obtained reveal that applied at a concentration range of 1-100 μg/ml for 24 – 72h disulfiram significantly decreases viability and proliferation of LSR-SF-SR rat sarcoma cells in a time- and concentration-dependent manner (**Fig. 1A, Table 1**). The cytotoxic activity of DS has been confirmed by MTT test (that measures the ability of the mitochondrial enzyme succinate dehydrogenase to convert yellow thiazolyl blue tetrazolium bromide into purple formazane) – accepted as a gold standard for cytotoxicity assays [14] and staining with crystal violet dye that binds to proteins and DNA [5] (**Fig. 1B, Table 1**). Cytopathological alternations such as cellular polymorphism are observed in LSR-SF-SR cells cultured for 72 h in the presence of DS applied at concentrations of 0.39 to 25 μg/ml. Individual cell size varies up to 4-5 times. Giant cells (of about 30 μm in diameter) and large optically dense cytoplasmic vacuoles have been observed. The nuclei are intact and multiple nucleoli (2 or more) are visible. Cytoplasmic morphology is erased. Apoptotic changes of the cell membrane are found only in single cells with dimensions less than 10 μm. Mitotic activity is missing (**Fig. 2**).

The rapid cytotoxic effect of disulfiram observed after short treatment intervals (24-72h) has been found to persist over time – the long-term experiments (30 days) carried out by CFM show that administered at concentrations ≥ 3.12 μg/ml DS completely inhibits 3D growth of rat sarcoma cells in a semisolid medium.



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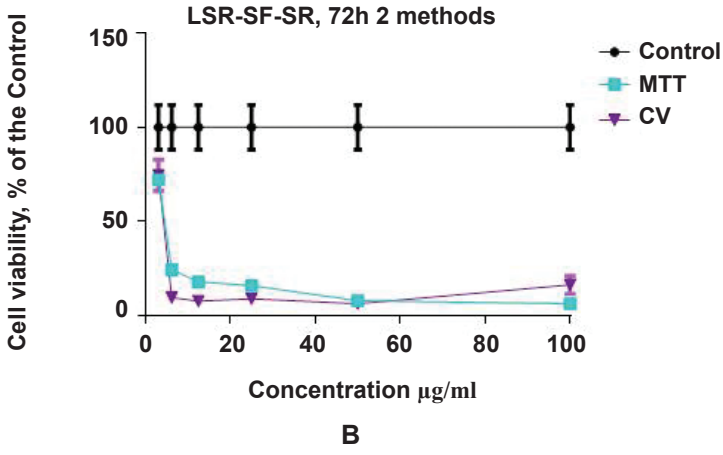

Fig.1 Effect of disulfiram on viability and proliferation of LSR-SF-SR rat sarcoma cells. The investigations are performed by MTT test (MTT) after 24, 48 and 72 h of treatment (Fig. 1A) as well as by MTT test and crystal violet staining (CV) after 72 h incubation period (Fig. 1B).

Table 1. Cytotoxic concentrations 50 (CC_{50}, μg/ml) and 90 (CC_{90}, μg/ml) of disulfiram for LSR-SF-SR rat sarcoma cells determined by MTT test (MTT) and crystal violet staining (CV)

Cytotoxicity assay	**MTT**			**CV**
Treatment period	24h	48h	72h	72h
CC_{50}	42.1	27.2	4.4	4.3
CC_{90}	n.d.	85.4	45.0	6.24

n.d. – not determined

Discussion

Disulfiram has been used for the treatment of chronic alcohol dependence since 1951. The promising anticancer properties of DS have also been reported – DS induces apoptosis in cancer cells, inhibits their proliferation and dissemination (metastases). Various mechanisms clarifying at least partially the antineoplastic potential of DS have been suggested including inhibition of proteasome functions and topoisomerase and matrix metalloproteinase activity, ability to suppress cancer STAT3 signaling, inhibition of TGF-beta-induced epithelial mesenchymal transition via ERK/NF-kB/Snail pathway, etc. Important feature of DS's anticancer activity is its ability to suppress cancer stem cells. DS has the potential to enhance the activity of anticancer agents and to overcome drug resistance (for example to alkylating agents) [6, 7, 8, 10, 12]. The protective effect of DS against the doxorubicin-induced cardiotoxicity has been documented [13]. The cytotoxicity of disulfiram is copper-dependent. The thiol groups

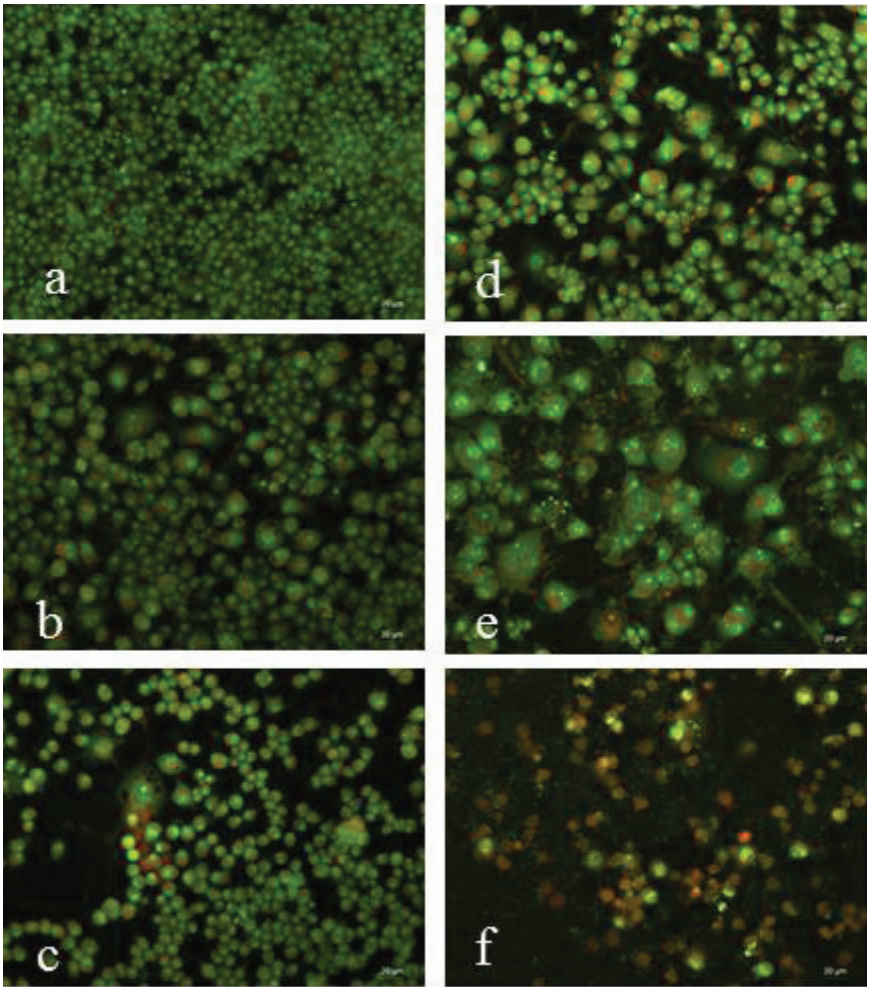

Fig. 2. Non-treated (Control) LSR-SF-SR rat sarcoma cells (a) and cells cultured in the presence of disulfiram applied for 72h at a concentration of 0.39 (b), 3.12 (c), 6.25 (d), 12.5 (e) and 25(f) μg/ml. Double staining with acridine orange and propidium iodide.

and thiuram structure has been proved to be indispensable for the anticancer activity of DS [3]. In this study we report for the first time that DS decreases 2D- and 3D-growth of retrovirus transformed rat sarcoma cells containing *v-src* gene. Src family kinases are known to play an important role in tumor development / progression being involved in cell adhesion, invasion, proliferation, survival, and angiogenesis and have been recognized as promising therapeutic targets for cancer [9].

Conclusion

The promising antitumor properties of disulfiram as well as its well known pharmacokinetics, pharmacodynamics and toxicity profile make it a suitable candidate for

the so called “drug repurposing” – a promising strategy for cancer therapy. Additional studies are needed to clarify better the antitumor activity of disulfiram and to establish the most appropriate form of its application.

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